EXHIBIT A

CLINICAL THERAPEUTICS®/VOL. 24, NO. 7, 2002

Pharmacokinetics and Pharmacodynamics of Piperacillin/Tazobactam When Administered by Continuous Infusion and Intermittent Dosing

*David S. Burgess, PharmD, and Travis Waldrep, PharmD**

College of Pharmacy, University of Texas at Austin, and Departments of Pharmacology and Medicine, University of Texas Health Science Center at San Antonio, Texas

ABSTRACT

Background: Although intermittent bolus dosing is currently the standard of practice for many antimicrobial agents, beta-lactams exhibit time-dependent bacterial killing. Maximizing the time above the minimum inhibitory concentration (MIC) for a pathogen is the best pharmacodynamic predictor of efficacy. Use of a continuous infusion has been advocated for maximizing the time above the MIC compared with intermittent bolus dosing.

Objective: This study compared the pharmacokinetics and pharmacodynamics of piperacillin/tazobactam when administered as an intermittent bolus versus a continuous infusion against clinical isolates of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Methods: Healthy volunteers were randomly assigned to receive piperacillin 3 g/tazobactam 0.375 g q6h for 24 hours, piperacillin 6 g/tazobactam 0.75 g continuous infusion over 24 hours, and piperacillin 12 g/tazobactam 1.5 g continuous infusion over 24 hours. Five clinical isolates each of *P aeruginosa* and *K pneumoniae* were used for pharmacodynamic analyses.

Results: Eleven healthy subjects (7 men, 4 women; mean ± SD age, 28 ± 4.7 years) were enrolled. Mean steady-state serum concentrations of piperacillin were 16.0 ± 5.0 and 37.2 ± 6.8 μg/mL with piperacillin 6 and 12 g, respectively. Piperacillin/tazobactam 13.5 g continuous infusion (piperacillin 12 g/tazobactam 1.5 g) was significantly more likely to produce a serum inhibitory titer ≥1:2 against *P aeruginosa* at 24 hours than either the 6.75 g continuous infusion (piperacillin 6 g/tazobactam 0.75 g) or 3.375 g q6h (piperacillin 3 g/tazobactam 0.375 g). There were no statistical differences against *K pneumoniae* between

This paper was presented at the annual American College of Clinical Pharmacy meeting in Los Angeles, California, on November 7, 2000.

*Current affiliation: Department of Pharmacy, Parkland Hospital, Dallas, Texas.

Accepted for publication May 8, 2002.

0149-2918/02/$19.00

regimens. The median area under the inhibitory activity–time curve (AUIC) for the 13.5 g continuous infusion was higher than that for 3.375 g q6h and the 6.75 g continuous infusion against both *P aeruginosa* and *K pneumoniae* ($P \leq 0.007$, 13.5 g continuous infusion and 3.375 g q6h vs 6.75 g continuous infusion against *K pneumoniae*). The percentage of subjects with an AUIC ≥125 was higher with both 3.375 g q6h and the 13.5 g continuous infusion than with the 6.75 g continuous infusion against *P aeruginosa* and *K pneumoniae* (both, $P < 0.001$ vs 6.75 g continuous infusion against *K pneumoniae*).

Conclusions: Piperacillin 12 g/tazobactam 1.5 g continuous infusion consistently resulted in serum concentrations above the breakpoint for Enterobacteriaceae and many of the susceptible strains of *P aeruginosa* in this study in 11 healthy subjects. Randomized controlled clinical trials are warranted to determine the appropriate dose of piperacillin/tazobactam.

Key words: piperacillin/tazobactam, continuous infusion, pharmacodynamics, pharmacokinetics. (*Clin Ther.* 2002;24:1090–1104)

INTRODUCTION

The pharmacodynamic principles of antimicrobial agents can be separated into 2 main categories: concentration dependent (ie, aminoglycosides and fluoroquinolones) and concentration independent, or time dependent (ie, beta-lactams and vancomycin).[1] The rate and extent of killing with concentration-dependent agents are maximized when the ratio of maximum concentration (C_{max}) to minimum inhibitory concentration (MIC) is from 8:1 to 10:1.[2] Concentration-dependent antimicrobial agents rely on a postantibiotic effect, or continued suppression of bacterial regrowth after exposure.[3] However, beta-lactams (except carbapenems) exhibit a brief or no postantibiotic effect against gram-negative organisms. In fact, the rate and extent of killing with these agents are maximized when the duration of the drug concentration at the site of infection is maximized. Specifically, there is no greater bacterial killing once the ratio of serum concentration to MIC reaches ~4 to 5 times the MIC.[4-6] An area under the inhibitory activity–time curve (AUIC) ≥125 is another pharmacodynamic parameter that appears to correlate with clinical success with beta-lactams against gram-negative organisms.[7]

Intermittent bolus dosing is currently the standard of practice for many antimicrobial agents. Continuous infusions of beta-lactams have been advocated for maximizing the time the antimicrobial concentration remains above the MIC compared with intermittent bolus dosing. However, there are limited clinical data describing the clinical efficacy of a continuous infusion compared with an intermittent bolus regimen.[8-10]

The objectives of this study were to compare the pharmacokinetics and pharmacodynamics of piperacillin/tazobactam* when administered as an intermittent bolus versus a continuous infusion against clinical isolates of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The pharmacodynamic properties of continuous versus bolus dosing were determined by comparison of serum inhibitory titers (SITs) and serum bactericidal titers (SBTs), as well as the AUIC and the area under the bactericidal activity–time curve (AUBC).

*Trademark: Zosyn® (Wyeth Pharmaceuticals, Philadelphia, Pennsylvania).

SUBJECTS AND METHODS

Subjects

Volunteers between the ages of 18 and 35 years were eligible to participate after undergoing a complete medical history, physical examination, and laboratory screening. Patients were excluded based on the following: history of allergy to beta-lactams, history of drug or alcohol abuse, history or evidence of any chronic disease (eg, HIV infection, hypertension, diabetes, asthma), evidence of hepatic impairment, or creatinine clearance <80 mL/min (as calculated using the method described by Cockcroft and Gault[29]). Additional exclusion criteria were pregnancy and lactation. Subjects were required to abstain from alcohol or nicotine-containing products during the study.

This study was approved by the US Food and Drug Administration and by the University of Texas Health Science Center at San Antonio, South Texas Veterans Health Care System, and Frederic C. Bartter General Clinical Research Center, all in San Antonio, Texas. All subjects provided written informed consent before enrollment.

Study Design

This was a crossover study in which each subject received piperacillin 3 g/tazobactam 0.375 g IV over 30 minutes q6h for 24 hours (total daily dose with intermittent dosing: piperacillin 12 g/tazobactam 1.5 g), piperacillin 6 g/tazobactam 0.75 g continuous infusion over 24 hours (half of the total daily dose with intermittent dosing), and piperacillin 12 g/tazobactam 1.5 g continuous infusion over 24 hours. The order of administration was randomly determined. Each subject was admitted for 24 hours to the General Clinical Research Center at the Audie L. Murphy Veterans Affairs Hospital, San Antonio, on 3 separate occasions. A washout period of ≥7 days was required between receipt of the regimens.

Antimicrobial Administration

Piperacillin/tazobactam was reconstituted according to the manufacturer's recommendations and further diluted with dextrose 5% water to a volume of 100 or 1000 mL for the intermittent and continuous infusions, respectively. All administrations were through a peripheral venous catheter. The intermittent bolus was infused over 30 minutes, whereas the continuous infusions were given at a rate of 250 mg/h for piperacillin 6 g and 500 mg/h for piperacillin 12 g over the 24-hour period.

Blood Sampling

Blood samples for pharmacokinetic analysis were obtained from a peripheral venous catheter in the opposite arm to that used for drug infusion. For the intermittent bolus regimen, samples were obtained at the following times: before the start of administration ($t = 0$) and at 18, 18.5 (peak), 19, 20, 21 (midpoint), and 24 hours (trough). For both continuous infusion regimens, samples were obtained at the following times: before the start of administration ($t = 0$) and at 0.5, 1, 2, 4, 6, 8, 12, 18, and 24 hours after the start of the infusion. Additional samples were obtained at the following times for the pharmacodynamic analysis: 19 hours (peak for intermittent regimen), 21 hours (midpoint for intermittent regimen), and 24 hours (trough for intermittent regimen)

after the start of the infusion. After sampling, the blood was allowed to clot and was centrifuged for 10 minutes at 1000*g*. The supernatant was stored at −70°C until assayed or used for the microbiologic analysis.

Piperacillin and Tazobactam Analysis

Piperacillin and tazobactam concentrations in serum were analyzed simultaneously at the University of Texas Health Science Center at San Antonio by reverse-phase high-performance liquid chromatography (HPLC), as has been previously described.[16] The chromatographic equipment consisted of 510 HPLC pumps, 717 Autosampler, 486 Tunable Absorbance Detector, Waters System Interface Module (all, Waters Corporation, Milford, Mass), Brownlee C18 column (15 cm × 3.2 mm), and C18 Guard Pak (both, Alltech Associates, Deerfield, Ill). Mobile phase A consisted of 97% 0.01 mol/L sodium phosphate and 3% acetonitrile, mobile phase B was 70% 0.01 mol/L sodium phosphate and 30% acetonitrile. The flow rate was 1.5 mL/min. The chromatograms were generated with a linear gradient program of 95% eluent A and 5% eluent B to 50% eluent A and 50% eluent B in 9 minutes and a final linear step of 95% eluent A and 5% eluent B in 1 minute. Total run time was set at 17 minutes. The wavelength for detection was 220 nm.

Piperacillin and tazobactam standards were prepared in pooled human serum. Proteins were precipitated by adding 800 µL of acetonitrile to 200 µL of serum and 200 µL of 0.05 mol/L sodium phosphate buffer at a pH of 6.0. The samples were then vortexed for 30 seconds and centrifuged at 6000 rpm for 15 minutes. The resultant supernatant was removed and transferred to a glass test tube to which 2 mL of dichloromethane was added. Each tube was then vortexed for 30 seconds and centrifuged for 15 minutes. The upper aqueous layer was then transferred to an autosampler vial and 50 µL injected in duplicate on the column. The retention times for tazobactam and piperacillin were 6 and 12 minutes, respectively. The plot was linear over the concentration range from 1 to 200 µg/mL for piperacillin ($r^2 \geq 0.998$) and tazobactam ($r^2 \geq 0.997$). The interday coefficients of variation were <4% for both piperacillin and tazobactam. The interday coefficients of variation were <2% and 4% at all concentrations of piperacillin and tazobactam, respectively.

Pharmacokinetic Analysis

Noncompartmental methods were used for the calculation of all pharmacokinetic variables of piperacillin and tazobactam. For intermittent dosing, the elimination half-life ($t_{1/2}$) was calculated as $\ln 2/\lambda_z$, where λ_z is the absolute value of the slope of the least squares regression line for ≥3 terminal data points. The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal method. The total body clearance (TBCl) was calculated as TBCl = dose/AUC. The volume of distribution at steady state (V_{ss}) was calculated using the equation V_{ss} = TBCl/λ_z.

For the continuous infusions, $t_{1/2}$ was calculated as $t_{1/2} = 0.693\ V_{ss}/TBCl$. TBCl was calculated as $TBCl = Ko/C_{ss}$, where Ko is the infusion rate and C_{ss} is the steady-state plasma concentration. V_{ss} was calculated using the following equation: $V_{ss} = [(Ko \cdot T) - (TBCl \cdot AUC)]/C_{ss}$, where Ko is the infusion rate, T is the duration of the infusion, C_{ss} is the steady-state plasma concentration, and TBCl is the

total body clearance. The AUC was calculated using the trapezoidal and log-trapezoidal methods.

Microbiologic Analysis

The in vitro activity of piperacillin/tazobactam was determined against 5 clinical isolates each of *K pneumoniae* and *P aeruginosa*. The isolates were selected to provide a range of MICs for piperacillin/tazobactam. MICs were determined in triplicate using the broth microdilution technique, according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines.[17] A stock solution of piperacillin was prepared immediately before testing. The range of concentrations studied was 0.25 to 512 μg/mL for piperacillin. The concentration of tazobactam was fixed at 4 μg/mL. Sterile microdilution trays were used containing a total volume of 100 μL of test medium per well. In vitro activity was determined in Mueller-Hinton broth supplemented with calcium chloride (50 mg/L) and magnesium sulfate (12.5 mg/L). The final inoculum, prepared according to NCCLS guidelines, was verified using the Spiral® Plater (Spiral Biotech, Bethesda, Md). MIC was defined as the lowest concentration of antibiotic that prevented turbidity, as detected by the unaided eye.

Pharmacodynamic Analysis

SITs were determined in duplicate using the broth microdilution technique, according to NCCLS guidelines.[18] SITs and SBTs were determined at 19, 21, and 24 hours after the start of administration. All samples were diluted with 50% Mueller-Hinton broth and 50% human serum in 2-fold steps from 1:2 to 1:1024 in 96-well microtiter plates. Each test well contained 50 μL of antibiotic and 50 μL of inoculum. The final inoculum contained ~10^5 colony-forming units (CFUs)/mL and was verified using the Spiral Plater. The plates were incubated for 18 to 24 hours at 35°C. The SIT was defined as the highest dilution that showed no visible turbidity. A 10-μL sample from each well showing no visible growth was subcultured onto Mueller-Hinton agar and incubated for 24 hours at 35°C. SBTs were determined by identifying the largest dilution that resulted in a 99.9% reduction in CFUs compared with the initial inoculum. Before determination of the activity in each subject serum sample, isolates were screened against drug-free (predose) serum from each of the subjects to document lack of growth inhibition secondary to unidentified serum inhibitory factors.

Mean inhibitory and bactericidal titers at each time point were determined by assigning an ordinal number to each reciprocal titer (eg, <1:2, 0; 1:2, 1; ... 1:512, 10). These ordinal numbers were averaged for each subject, organism, regimen, and sampling time and rounded to the nearest whole number. Mean values were then reconverted to the corresponding reciprocal inhibitory or bactericidal titer.

The AUIC and AUBC were calculated from the inverse plasma inhibitory and bactericidal titers at different time points after antibiotic administration using the trapezoidal rule. For intermittent bolus dosing, AUIC and AUBC from 0 to 24 hours ($AUIC_{0-24}$ and $AUBC_{0-24}$) were calculated by multiplying the number of doses given per day by the AUIC and AUBC. Median 24-hour AUIC and AUBC ratios were calculated for each regimen and isolate.

Statistical Analysis

Differences in the pharmacokinetic parameters, SIDs/SBTs, and AUIC/AUBC were determined by analysis of variance with the Scheffé post hoc test. A P value <0.05 was considered statistically significant.

RESULTS

Subjects

Eleven healthy subjects (7 men, 4 women) were enrolled in the study. Subjects' mean (±SD) age, serum creatinine level, and estimated creatinine clearance were 28 ± 4.7 years, 1.0 ± 0.2 mg/dL, and 97 ± 11 mL/min, respectively. All piperacillin/tazobactam regimens were well tolerated, with the exception of mild diarrhea in 2 subjects after receipt of the bolus regimen.

Pharmacokinetics

The mean (±SD) C_{max} of the piperacillin intermittent bolus was 179.3 ± 43.5 μg/mL. The mean steady-state serum concentrations of piperacillin after continuous infusion of 6 and 12 g were 16.0 ± 5.0 and 37.2 ± 6.8 μg/mL, respectively. The AUC_{0-24} of piperacillin was significantly lower for the 6 g continuous infusion (330 ± 109 mg/L·h) than for either the 12 g continuous infusion (731 ± 140 mg/L·h) or the intermittent bolus (926 ± 162 mg/L·h) ($P < 0.001$). No statistical differences were detected between regimens for the mean TBCl of piperacillin (intermittent bolus, 13.3 ± 2.3 L/h; piperacillin 12 g, 13.9 ± 2.9 L/h; piperacillin 6 g, 17.1 ± 5.2 L/h).

For the tazobactam intermittent infusion, the peak concentration was 14.5 ± 1.8 μg/mL. The steady-state concentration of tazobactam was 2.3 ± 0.65 μg/mL with the 1.5 g continuous infusion. However, with the lower dose of tazobactam (0.75 g continuous infusion), serum concentrations were <1 μg/mL, which was the lower limit of detection for the assay.

Susceptibility Testing

The MICs for each of the 5 isolates are presented in Table I. *K pneumoniae* and *P aeruginosa* MICs ranged from 2/4 to 32/4 μg/mL and 4/4 to 64/4 μg/mL, respectively. The NCCLS breakpoints for piperacillin/tazobactam are ≤64/4 μg/mL for *P aeruginosa* and ≤16/4 μg/mL for

Table I. In vitro activity of piperacillin/tazobactam.

Pseudomonas aeruginosa		*Klebsiella pneumoniae*	
Isolate	MIC, μg/mL	Isolate	MIC, μg/mL
99-032	4/4	99-009	2/4
99-012	8/4	99-010	4/4
99-026	16/4	99-011	8/4
99-017	32/4	99-004 (ESBL)	8/4
99-023	64/4	99-015	32/4

MIC = minimum inhibitory concentration; ESBL = extended-spectrum beta-lactamase.

K pneumoniae, including extended-spectrum beta-lactamase-producing (ESBL) species. All *P aeruginosa* isolates and 4 of 5 *K pneumoniae* isolates were susceptible to piperacillin/tazobactam. One *K pneumoniae* isolate was an ESBL organism with an MIC of 8/4 μg/mL.

Pharmacodynamics

Piperacillin 3 g/tazobactam 0.375 g q6h provided serum concentrations above the MIC for ≥60% of the dosing interval against *P aeruginosa* and *K pneumoniae* in 40% and 80% of subjects, respectively. In fact, intermittent bolus dosing provided serum concentrations above the MIC for ≥60% of the dosing interval against organisms with an MIC ≤8 μg/mL. As shown in Table II, piperacillin steady-state concentrations after administration of piperacillin 12 and 6 g continuous infusion were above the MIC for 76% and 48% of subjects, respectively, against *P aeruginosa* and 96% and 80% of subjects against *K pneumoniae*. As the desired serum concentration increased to 4 times the MIC, the percentage of subjects achieving that concentration decreased to 36% and 8% against *P aeruginosa* and 72% and 28% against *K pneumoniae* for both the high and low doses of continuous-infusion piperacillin.

For organisms with an MIC ≤8 μg/mL or ≤32 μg/mL, low- and high-dose piperacillin/tazobactam continuous infusion will provide concentrations above the MIC for ≥80% of subjects. If the desired steady-state concentrations are 4 times the MIC, then the MICs must be ≤2 and ≤8 μg/mL for low- and high-dose continuous-infusion piperacillin, respectively (Table III).

Piperacillin/tazobactam 13.5 g continuous infusion (piperacillin 12 g/tazobactam 1.5 g) was statistically significantly more likely to produce an STT ≥1:2 against *P aeruginosa* at 24 hours than either 6.75 g (piperacillin 6 g/tazobactam 0.75 g) continuous infusion or 3.375 g (piperacillin 3 g/tazobactam 0.375 g) q6h ($P = 0.024$). For *P aeruginosa*, 74% of subjects receiving the 13.5 g continuous infusion regimen maintained STTs ≥1:2 at 24 hours, compared with 47% and 62% of the 3.375 g intermittent bolus and 6.75 g continuous infusion regimens, respectively. For *K pneumoniae*, 100% of subjects receiving

Table II. Pharmacodynamics of continuous infusion piperacillin against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.*

Organism	No. (%) of Subjects with Steady-State Piperacillin Concentrations Above MIC			
	≥1 Time	≥2 Times	≥3 Times	≥4 Times
P aeruginosa	76 (48)	56 (28)	40 (18)	36 (8)
K pneumoniae	96 (88)	80 (56)	80 (40)	72 (28)

MIC = minimum inhibitory concentration.
*The first number is the proportion of subjects receiving a 12 g continuous infusion of piperacillin. The number in parentheses is the proportion of subjects receiving a 6 g continuous infusion of piperacillin.

Table III. Percentage of subjects with steady-state concentrations at specified multiples of the minimum inhibitory concentration (MIC) after receiving piperacillin 12 and 6 g by continuous infusion.*

MIC, μg/mL	No. (%) of Subjects with Stated Serum Piperacillin Concentrations Above MIC			
	≥1 Time	≥2 Times	≥3 Times	≥4 Times
2	100 (100)	100 (100)	100 (100)	100 (100)
4	100 (100)	100 (100)	100 (80)	100 (40)
8	100 (100)	100 (40)	100 (10)	80 (0)
16	100 (40)	80 (0)	0 (0)	0 (0)
32	80 (0)	0 (0)	0 (0)	0 (0)
64	0 (0)	0 (0)	0 (0)	0 (0)

*The first number is the proportion of subjects receiving a 12 g continuous infusion of piperacillin. The number in parentheses is the proportion of subjects receiving a 6 g continuous infusion of piperacillin.

the 13.5 g continuous infusion maintained STTs ≥1:2 at 24 hours, compared with 87% and 98% of those receiving the 3.375 g intermittent bolus and 6.75 g continuous infusion regimens, respectively. There were no statistical differences between regimens against *K pneumoniae*. The percentage of subjects with STTs ≥1:2 is shown in Figure 1.

The median AUIC for the 13.5 g continuous infusion was higher than that for 3.375 g q6h and the 6.75 g continuous infusion against both *P aeruginosa* and *K pneumoniae* ($P \leq 0.007$, 13.5 continuous infusion and 3.375 g q6h vs 6.75 g continuous infusion against *K pneumoniae*) (Figure 2). However, none of the regimens achieved median AUICs ≥125 against *P aeruginosa*. For *K pneumoniae*, the 13.5 g continuous infusion was the only regimen to achieve a median AUIC ≥125. The median AUIC was the same for all 3 regimens against *P aeruginosa*, whereas the 13.5 g continuous infusion and 3.375 g q6h produced a statistically larger AUIC than did the 6.75 g continuous infusion against *K pneumoniae* ($P \leq 0.002$). The percentage of subjects with an AUIC ≥125 was higher with both 3.375 g q6h and the 13.5 g continuous infusion than with the 6.75 g continuous infusion against both *P aeruginosa* and *K pneumoniae* (both, $P < 0.001$ vs 6.75 g continuous infusion against *K pneumoniae*) (Figure 3).

DISCUSSION

The efficacy of any antimicrobial regimen depends on the interplay of a variety of bacterial, drug, and host factors. The pharmacodynamics of antimicrobial agents relate clinically achievable concentrations at the site of infection (pharmacokinetics) to the antimicrobial effects of the agent (MIC). The best pharmacodynamic predictor of efficacy for the beta-lactams is the amount of time the serum concentration remains above the MIC. Because most beta-lactam antibiotics have short

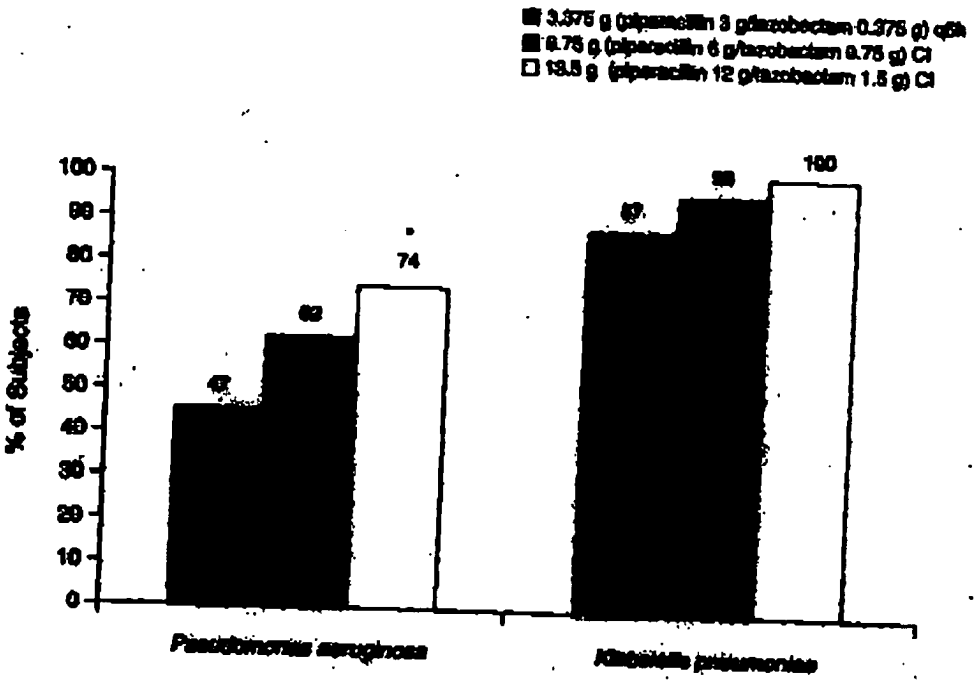

Figure 1. Percentage of subjects with serum inhibitory titers ≥1:2 at 24 hours with 3 dosing regimens of piperacillin/tazobactam. CI = continuous infusion. $^{*}P = 0.024$ vs 3.375 g q6h and 6.75 g CI.

half-lives (ie, 1-2 hours), frequent administration of bolus doses is needed to maintain serum concentrations above the MIC. As the MIC of the pathogen increases, maintaining serum concentrations above the MIC for the entire dosing interval becomes more challenging. However, data from animal studies have demonstrated that beta-lactams do not need to be above the MIC for the entire dosing interval to have maximal effect.[1,19] The maximal effect against gram-negative organisms is observed when the serum concentration remains above the MIC for 60% to 70% of the dosing interval. For gram-positive organisms, the serum concentration needs to be above the MIC for only 40% to 50% of the dosing interval. This difference in time above the MIC for gram-positive and gram-negative organisms is due to the postantibiotic effect displayed by beta-lactams against gram-positive bacteria.

Continuous infusion is a mode of administration that can maintain concentrations above the MIC for the entire dosing interval. However, which concentration should be targeted during continuous infusion of beta-lactams has not yet been established fully. Should the target concentration be the MIC or some multiple of the MIC? Results from time-kill curves and in vitro models demonstrate that maximal killing of gram-negative bacteria occurs at 4 times the MIC for beta-lactams.[1,3,20] Furthermore, in vitro studies have demonstrated that a steady-state con-

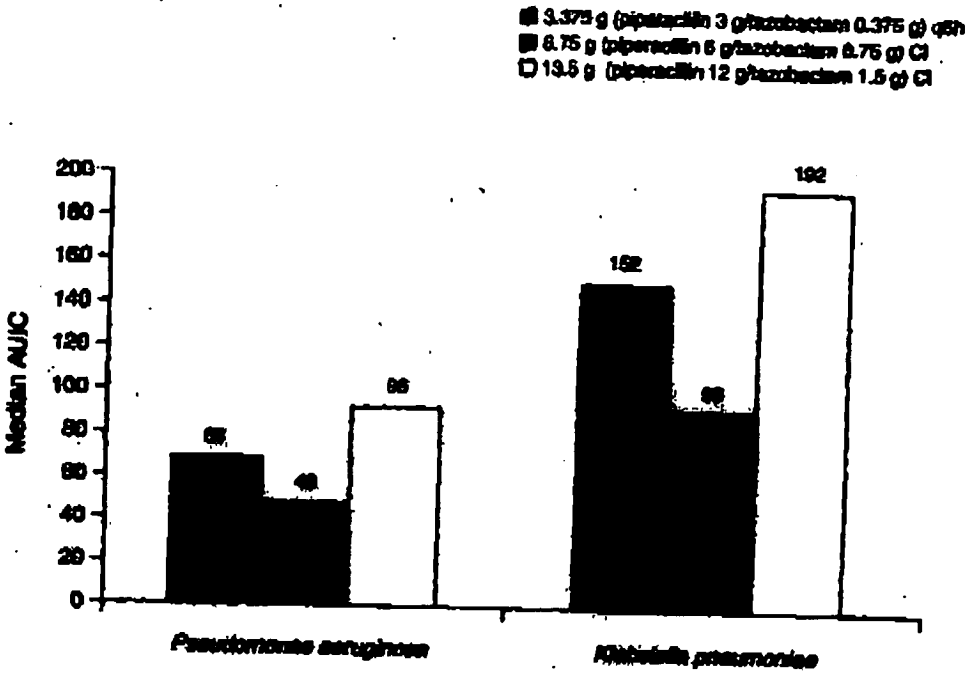

Figure 2. Median area under the inhibitory activity–time curve (AUIC) for 3 dosing regimens of piperacillin/tazobactam. CI = continuous infusion.

centration at the MIC allows the organism to regrow and develop resistance.[2,20] Hence, a good target concentration for continuous infusion of beta-lactams appears to be 4 times the MIC and not just above the MIC. However, in vivo dose-ranging studies have not been performed for continuous infusion regimens. Rocaendaal et al[21,22] assessed the administration of ceftazidime by continuous infusion and intermittent bolus against *K pneumoniae* pneumonia in normal and leukopenic rats. The findings of these studies demonstrated that not only should the MIC be considered but also the severity of disease and host defenses. In normal rats, the concentration of ceftazidime by intermittent bolus to protect 100% of the animals was one third the MIC for moderate infections and about 6 times the MIC for severe infections. In neutropenic animals with moderate infections, the required concentration was 2 times the MIC for 100% survival. Furthermore, depending on the immune status of the animals, the intermittent bolus required 4 to 16 times more drug than continuous infusion to achieve the same effect. Potential advantages of continuous infusion are maximization of the pharmacodynamic profile with a lower total daily dose, fewer adverse effects, and substantial savings in antibiotic cost.[9,10] Currently, clinical data on the efficacy of continuous infusions are limited. However, trials comparing continuous infusions with intermittent

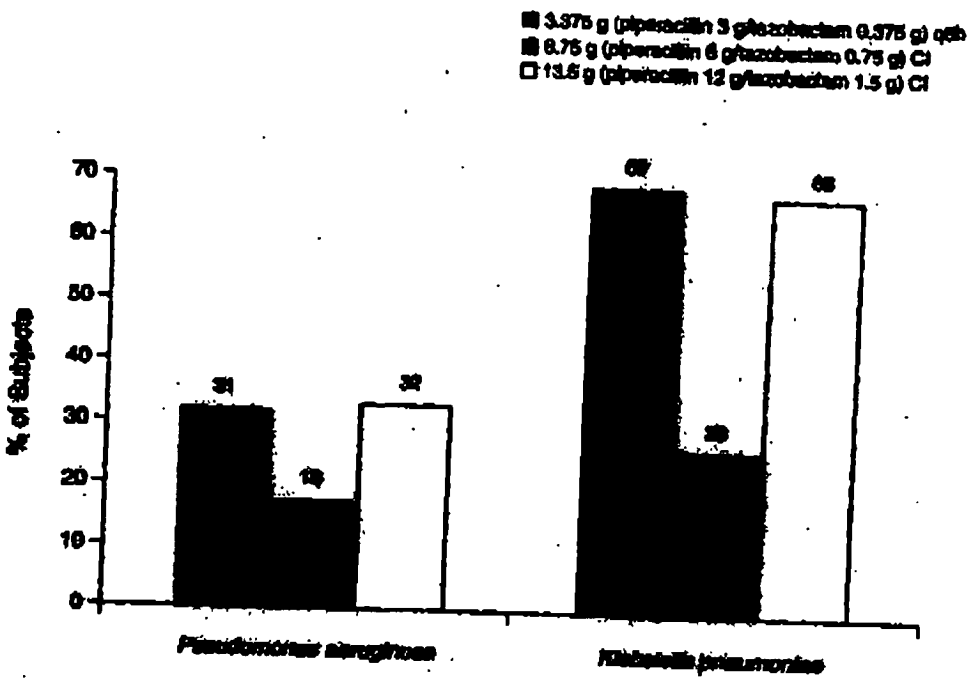

Figure 3. Percentage of subjects with area under the inhibitory activity–time curve ≥125 with 3 dosing regimens of piperacillin/tazobactam. CI = continuous infusion.

doses of azlocillin,[30] carbenicillin,[9] cefmetazole,[9,11,14,29-31] cefepime,[31] cefuroxime,[10,32] piperacillin/tazobactam,[33] ticarcillin/clavulanate,[34] and ureidopenicillins[35] have been performed in healthy subjects as well as in some critically ill patients.

There have been few studies assessing the pharmacokinetics of piperacillin/tazobactam administered by continuous infusion.[33] Richenson et al[33] assessed the pharmacokinetics of piperacillin/tazobactam administered by continuous infusion or intermittent bolus with once-daily gentamicin in healthy volunteers. Mean (±SD) serum steady-state concentrations of piperacillin at 500 mg/h continuous infusion were 28.0 ± 6.9 μg/mL and were unchanged with gentamicin administration. Similarly, the present study found mean serum steady-state concentrations after administration of 12 and 6 g piperacillin to be 37.2 ± 6.8 μg/mL and 16.0 ± 3.0 μg/mL, respectively. Serum steady-state concentrations of piperacillin ranged from 9.4 to 24.9 μg/mL and 25.8 to 47.5 μg/mL for the 6- and 12-g dosing regimens, respectively. The pharmacokinetic profile of the intermittent bolus of piperacillin/tazobactam was similar to that reported by other investigators.

Data on the in vitro pharmacodynamics and clinical efficacy of continuous infusion piperacillin/tazobactam are extremely limited.[36] Hence, it is currently necessary to assess the pharmacodynamic profile of continuous infusion piperacillin/

tazobactam in healthy volunteers. In the present study, continuous infusions of piperacillin 12 g/tazobactam 1.5 g and piperacillin 6 g/tazobactam 0.75 g resulted in mean (±SD) steady-state serum piperacillin concentrations of 37.2 ± 6.8 and 16.0 ± 5.0 μg/mL, respectively. Because the MIC for the majority of gram-negative bacteria is ≤8/4 μg/mL with piperacillin/tazobactam, steady-state concentrations are ≥4 times the MIC for piperacillin 12 g/tazobactam 1.5 g continuous infusion.[37] Even piperacillin 6 g/tazobactam 0.75 g will produce serum concentrations of 1 time the MIC for those organisms with an MIC ≤8/4 μg/mL. Furthermore, for more serious infections, as with *P. aeruginosa*, the combination of piperacillin with an aminoglycoside or fluoroquinolone would be recommended; we, as well as other investigators, have reported that piperacillin/tazobactam in combination with another antibiotic provides highly effective bacterial killing.[38,39]

Besides assessing the time serum concentration remains above the MIC, many investigators have suggested that AUC/MIC is an appropriate pharmacodynamic parameter not only for concentration-dependent antimicrobial agents such as the aminoglycosides and fluoroquinolones but also for time-dependent agents such as the beta-lactams.[40,41] In the present study, we found that the AUC/MIC was consistently higher against *K. pneumoniae* than against *P. aeruginosa* isolates and that continuous infusion piperacillin 12 g/tazobactam 1.5 g provided the highest AUC/MIC against both organisms. The higher AUC/MIC for *K. pneumoniae* compared with *P. aeruginosa* is due to the lower MICs for *K. pneumoniae*, which could be misleading given the fact that all of the *P. aeruginosa* isolates were susceptible to piperacillin/tazobactam, whereas 1 *K. pneumoniae* isolate was resistant to piperacillin/tazobactam. This difference in susceptibility is due to the different breakpoints for the 2 organisms. Hence, the use of combination therapy for the treatment of systemic infections caused by *P. aeruginosa* is warranted clinically, even if the organisms are classified as susceptible to piperacillin/tazobactam.

Integration of the pharmacokinetic profile and microbiologic activity is one way to assess the pharmacodynamics of antibiotics; however, this method does not account for host defenses or protein binding. The *SIT* takes these factors into account and was assessed in this study. For example, the piperacillin/tazobactam MIC was the same for 2 isolates of *K. pneumoniae* (8/4 μg/mL); however, the pharmacodynamic parameters (SITs and AUC/MIC) against these 2 isolates were extremely different. The reason for this difference was that 1 isolate was an ESBL organism; however, the ESBL organism was susceptible (MIC ≤8/4 μg/mL) to piperacillin/tazobactam based on NCCLS standards.[17] Several institutions, including our own, have demonstrated the benefit of piperacillin/tazobactam for decreasing the incidence of ESBL bacteria.[42] Further investigation and assessment of the pharmacodynamics of piperacillin/tazobactam against ESBL organisms are needed.

CONCLUSIONS

Clinically, the efficacy of beta-lactams appears to be maximized in dosing regimens that maximize the time above the MIC. This can best be accomplished by using continuous infusion. Furthermore, this mode of administration has the potential to be cost-effective. In this study in 11

volunteers, continuous infusion piperacillin 12 g/tazobactam 1.5 g consistently resulted in serum concentrations above the breakpoint for Enterobacteriaceae and many susceptible strains of *P aeruginosa*. Randomized, controlled clinical trials are warranted to determine the appropriate dose of piperacillin/tazobactam.

ACKNOWLEDGMENTS

This study was supported by an unrestricted research grant from Wyeth Pharmaceuticals, Philadelphia, Pennsylvania, and National Institutes of Health Grant RR-01346.

The authors acknowledge the nursing and dietetic care provided by the staff of the Frederic C. Bartter General Clinical Research Center at the South Texas Veterans Health Care System in San Antonio, Texas.

REFERENCES

1. Craig WA. Pharmacokinetic/pharmacodynamic parameters: Rationale for antibacterial dosing of mice and men. *Clin Infect Dis*. 1998;26:1–10.

2. Moore RD, Lietman PS, Smith CR. Clinical response to aminoglycoside therapy: Importance of the ratio of peak concentration to minimal inhibitory concentration. *J Infect Dis*. 1987;155:93–99.

3. Craig WA, Ebert SC. Killing and regrowth of bacteria in vitro: A review. *Scand J Infect Dis Suppl*. 1990;74:63–70.

4. Vogelman B, Gudmundsson S, Leggett J, et al. Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in an animal model. *J Infect Dis*. 1988;158:831–847.

5. Ryatt JM, McKinnon PS, Zimmer GS, Schentag JJ. The importance of pharmacokinetic/pharmacodynamic surrogate markers to outcome. Focus on antibacterial agents. *Clin Pharmacokinet*. 1995;28:143–160.

6. Leggett JE, Fantin B, Ebert S, et al. Comparative antibiotic dose-effect relations at several dosing intervals in murine pneumonitis and thigh-infection models. *J Infect Dis*. 1989;159:281–292.

7. Thomas JK, Forrest A, Bhavnani SM, et al. Pharmacodynamic evaluation of factors associated with the development of bacterial resistance in acutely ill patients during therapy. *Antimicrob Agents Chemother*. 1998;42:521–527.

8. Bodey GP, Kantarjian SJ, Rodriguez V. A randomized study of carbenicillin plus cefamandole or tobramycin in the treatment of febrile episodes in cancer patients. *Am J Med*. 1979;67:608–616.

9. Nicolas DP, McNabb J, Lacy MK, et al. Continuous versus intermittent administration of ceftazidime in intensive care unit patients with nosocomial pneumonia. *Int J Antimicrob Agents*. 2001;17:497–504.

10. Ambrose PG, Quintiliani R, Nightingale CH, Nicolau DP. Continuous vs. intermittent infusion of cefotaxime for the treatment of community-acquired pneumonia. *Infect Dis Clin Pract*. 1998;7:465–470.

11. Hanes SD, Wood GC, Herring V, et al. Intermittent and continuous ceftazidime infusion in critically ill trauma patients. *Am J Surg*. 2000;179:436–440.

12. Zeisler JA, McCarthy JD, Richelieu WA, Nichol MB. Cefuroxime by continuous infusion: A new standard of care? *Infect Med*. 1992;9:54–60.

13. Benko AS, Cappelletty DM, Kruse JA, Rybak MJ. Continuous infusion versus intermittent administration of ceftazidime in critically ill patients with suspected gram-negative infections. *Antimicrob Agents Chemother.* 1996;40:691–695.

14. Angus BJ, Smith MD, Suputtamongkol Y, et al. Pharmacokinetic-pharmacodynamic evaluation of ceftazidime continuous infusion versus intermittent bolus injection in septicaemic melioidosis. *Br J Clin Pharmacol.* 2000;49:445–452.

15. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron.* 1976;16:31–41.

16. Ozumgo AF, Hoyt KD, Wadgaonkar N, et al. Determination of tazobactam and piperacillin in human plasma, serum, bile and urine by gradient elution reversed-phase high-performance liquid chromatography. *J Chromatogr.* 1989;496:167–179.

17. National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard.* 4th ed. Villanova, Pa: NCCLS; 1997. Document M7-A4.

18. National Committee for Clinical Laboratory Standards. *Methodology for the Serum Bactericidal Tests; Approved Guidelines M21-A.* Villanova, Pa: NCCLS; 1999.

19. Craig WA. Interrelationship between pharmacokinetics and pharmacodynamics in determining dosage regimens for broad-spectrum cephalosporins. *Diagn Microbiol Infect Dis.* 1995;22:89–96.

20. Craig WA, Ebert SC. Continuous infusion of beta-lactam antibiotics. *Antimicrob Agents Chemother.* 1992;36:2577–2583.

21. Roosendaal R, Bakker-Woudenberg IA, van den Berg JC, Michel MF. Therapeutic efficacy of continuous versus intermittent administration of ceftazidime in an experimental *Klebsiella pneumoniae* pneumonia in rats. *J Infect Dis.* 1985;152:373–378.

22. Roosendaal R, Bakker-Woudenberg IA, van den Berghe-van Raffe M, Michel MF. Continuous versus intermittent administration of ceftazidime in experimental *Klebsiella pneumoniae* pneumonia in normal and leukopenic rats. *Antimicrob Agents Chemother.* 1986;30:403–408.

23. Burgess DS, Summers KK, Hardin TC. Pharmacokinetics and pharmacodynamics of aztreonam administered by continuous intravenous infusion. *Clin Ther.* 1999;21:1882–1889.

24. Lipman J, Gomersall CD, Gin T, et al. Continuous infusion ceftazidime in intensive care: A randomized controlled trial. *J Antimicrob Chemother.* 1999;43:309–311.

25. Daenen S, de Vries-Hospers H. Cure of *Pseudomonas aeruginosa* infection in neutropenic patients by continuous infusion of ceftazidime. *Lancet.* 1988;1:937. Letter.

26. Nicolau DP, Nightingale CH, Bansevicius MA, et al. Serum bactericidal activity of ceftazidime: Continuous infusion versus intermittent injections. *Antimicrob Agents Chemother.* 1996;40:61–64.

27. Bosso JA, Bosnjack CR, Flume PA, White RL. A pilot study of the efficacy of constant-infusion ceftazidime in the treatment of endobronchial infections in adults with cystic fibrosis. *Pharmacotherapy.* 1999;19:620–626.

28. Vinks AA, Brimicombe RW, Heijerman GH, Bakker W. Continuous infusion of ceftazidime in cystic fibrosis patients during home treatment: Clinical outcome, microbiology and pharmacokinetics. *J Antimicrob Chemother.* 1997;40:125–133.

29. Dannen S, Erjavec Z, Uges DR, et al. Continuous infusion of ceftazidime in febrile neutropenic patients with acute myeloid leukemia. *Eur J Clin Microbiol Infect Dis*. 1995;14:188–192.

30. Marshall E, Smith DB, O'Reilly SM, et al. Low-dose continuous infusion ceftazidime monotherapy in low-risk febrile neutropenic patients. *Support Care Cancer*. 2000;8:198–202.

31. Miyagawa CI, Andrie AM, Healy DP. Continuous ceftazidime infusions in critically ill surgical patients. *J Infect Dis Pharmacother*. 1999;4:25–34.

32. Burgess DS, Hastings RW, Hardin TC. Pharmacokinetics and pharmacodynamics of cefepime administered by intermittent and continuous infusion. *Clin Ther*. 2000; 22:66–75.

33. Richerson MA, Ambrose PG, Bui KQ, et al. Pharmacokinetic and economic evaluation of piperacillin/tazobactam administered either continuous or intermittent infusion with once-daily gentamicin. *Infect Dis Clin Pract*. 1999;8:195–200.

34. Bui KQ, Ambrose PG, Grant E, et al. Pharmacokinetics and pharmacoeconomic evaluation of ticarcillin-clavulanate administered as either continuous or intermittent infusion with once-daily gentamicin. *Infect Dis Clin Pract*. 1999;8:449–455.

35. Mouton JW, Michel MF. Pharmacokinetics of meropenem in serum and suction blister fluid during continuous and intermittent infusion. *J Antimicrob Chemother*. 1991;28:911–918.

36. Grant EM, Kuti JL, Nicolau DP, et al. Clinical efficacy and pharmacoeconomics of a continuous-infusion piperacillin/tazobactam program in a large community teaching hospital. *Pharmacotherapy*. 2002; 22:471–483.

37. Livermore DM, Carter MW, Bagel S, et al. In vitro activities of ertapenem (MK-0826) against recent clinical bacteria collected in Europe and Australia. *Antimicrob Agents Chemother*. 2001;45:1860–1867.

38. Burgess DS, Hastings RW. Activity of piperacillin/tazobactam in combination with amikacin, ciprofloxacin, and trovafloxacin against *Pseudomonas aeruginosa* by time-kill. *Diagn Microbiol Infect Dis*. 2000;38:37–41.

39. Owens RC Jr, Ramevicius MA, Nicolau DP, et al. In vitro synergistic activities of tobramycin and selected beta-lactams against 75 gram-negative clinical isolates. *Antimicrob Agents Chemother*. 1997;41: 2586–2588.

40. Smith PF, Ballow CH, Booker BM, et al. Pharmacokinetics and pharmacodynamics of azithromycin and telithromycin in hospitalized patients. *Clin Ther*. 2003;25:1231–1244.

41. Schentag JJ, Nix DE, Adelman MH. Mathematical examination of dose individualization principles (I): Relationships between AUIC above MIC and area under the inhibitory curve for cefmetazime, ciprofloxacin, and tobramycin. *DICP*. 1991; 25:1050–1057.

42. Peterson JE, Hardin TC, Kelly CA, et al. Association of antibiotic utilization measures and control of multiple-drug resistance in *Klebsiella pneumoniae*. *Infect Control Hosp Epidemiol*. 2000;21:455–458.

Address correspondence to: David S. Burgess, PharmD, Clinical Pharmacy Program–MSC 6220, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78229–3900. E-mail: burgessd@uthscsa.edu